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ARTICLE

Integrated Use of Organic and Bio-fertilizers to Improve Yield and Fruit Quality of Olives Grown in Low Fertility Sandy Soil in an Arid Environment

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ABSTRACT

Olive productivity should be improved through stimulating nutrition, particularly under poor fertility soils. Consequently, the objective of this study was to assess the efficacy of applying organic and bio-fertilizers on the physiological growth, yield and fruit quality of olive trees under newly reclaimed poor-fertility sandy soil in an arid environment. During a field experiment carried out at El-Qantara, North Sinai, Egypt over two consecutive seasons (2019–2020 and 2020–2021), olive Kalamata trees were evaluated under three organic fertilizer treatments alone or in combination with three bio-fertilizers treatments. Organic fertilizer was applied as goat manure (16.8 kg/tree/year), or olive pomace (8.5 kg/tree/year) in mid-December of each season *vs*. untreated trees. The bio-fertilizers were applied as N-fixing bacteria (150 g/tree) was inculated in early March of each season, or amino acid mixture (1.5%) was applied three times, at 70% of full bloom, 21 days after full bloom, and a month later in comparison to a non-fertilized trees (control). The cultivar used was Kalamata, a dual-purpose cultivar for oil and table olives whose value increases when processed as table olives. The results indicated that the goat manure followed by olive pomace significantly enhanced photosynthetic pigments (chlorophyll *a, b*, and carotenoids), leaf mineral contents (N, P, K, Ca, Mg and Fe), tree canopy volume, number of flowers per inflorescence, number of inflorescences per shoot, initial fruit set, fruit retention. For fruit quality, fruit length and width, fruit weight, and total fruit yield was increased compared to the non-fertilized control. Likewise, The bio-fertilizer N-fixing bacteria followed by the amino acid mixture significantly improved all of the aforementioned parameters. Accordingly, it is recommended, both environmentally and economically to utilize organic and bio-fertizers, particularly goat manure combined with N-fixing bacteria, in low-fertility soil to sustain olive production as well as reducing mineral fertilization.

KEYWORDS

Organic and bio-fertilizers; olives; kalamata; vegetative growth; leaf mineral contents; fruit quality

1 Introduction

Olives (*Olea europaea* L.) is an evergreen tree that belong to the family Oleaceae growing in a dry-subtropical climate [1]. It is a vital industrial crop in the Mediterranean region and has substantial economic and agricultural concerns [2]. The consumption of oil and table olives has gradually increased due to the fast-growing worldwide population. The total olives world cultivated area in 2022 was about 12.76 million hectares producing 23.64 million tonnes annually [3]. Egypt is involved in this statistic with 100,826 ha producing 932,927 tonnes with an average of 9,253 kg/ha [3]. Egypt is a major olive producer and a leader in olive cultivation in arid and semi-arid conditions, particularly in desert lands.

There are several olive cultivars for oil production or table olives, as well as several dual-purpose cultivars [4]. Kalamata is a dual-purpose cultivar of oil and table olives, but its value is higher when processed as table olives because it is considered as the best table olives in the world [5]. It is one of the most famous olive cultivars due to its attractive and deep reddish-purple fruits. Lately, the consumption of olive oil and table olives has gradually increased due to the fast-growing worldwide population. Hence, its productivity should be increased to reduce the gap between production and consumption.

Olive groves are commonly established in the newly reclaimed poor-fertility soils in the Mediterranean region [6]. The newly reclaimed soils have low biological and chemical properties as well as a lack of available mineral nutrients [7]. Consequently, agricultural production in newly reclaimed soils faces great challenges and yield low-crop productivity [8,9]. The deficiency of mineral nutrients is compensated by mineral fertilization. Olive tree growth depends on mineral components. According to the findings of a study by Yermiyahu et al. [10], the application of N and P may be advantageous because those nutrients' availability is determined by how vigorously olive trees blossom. K is seen as being very crucial in the production of olives [11], and there is a strong association between K and yield. Additionally, significant Ca requirements are needed for the flowering process and reproductive tissues; foliar treatments of this element in combination with Mg might directly affect yield, and this requirement probably increases throughout the flowering stage [12]. Different N levels were applied to the "Barnea" olive orchard, which resulted in a rise in the content of free fatty acids, an increase in palmitoleic, linoleic, and linolenic fatty acids, and a decrease in the level of polyphenols in the olive oil. on the other hand, oleic acid, the monounsaturated-to-polyunsaturated fatty acid ratio, and the oleic-to-linoleic ratio decreased. N, P, or K levels had no impact on the peroxide value [13]. Calcium spraying treatment during the growing season increased the oil content and the concentration of phenol compounds in fruits of Manzanilla de Sevilla and Ascolanta tenera olive cultivars [14,15].

Continuous application of chemical fertilization leads to the decline of soil fertility and properties and might increase heavy metals in plant materials, impacting the edibility and nutritional value [16]. On the other hand, sustainable agriculture aims to reduce mineral fertilization, a major global challenge. Hence, it has become essential to find alternative eco-friendly and economical sources of fertilizers to enhance poor soils.

Organic fertilization depends on using natural inputs such as decaying remains of organic matter, animal manure, and excess crops [17]. Organic fertilizer contains a high concentration of elements that can maintain soil fertility and secure sustainable nutrients for plant growth and productivity [18,19]. It improves soil fertility, structure, physical characteristics, and moisture retention [20]. Besides, it adjusts soil pH, aggregates soil stability, and provides plants with required minerals that are not mostly found in chemical fertilization [21]. Furthermore, it has a great advantage in sandy soil by increasing soil organic matter, reducing wind and water erosion, water-holding capacity, providing a source of slow-release nutrients, and promoting the growth of earthworms and other beneficial soil organisms [22,23]. Organic fertilization improves soil microorganisms, which play an essential role as a buffering agent against undesirable fluctuations of soil pH [24,25]. Organic fertilizers and nutrients are important to plants and can be used safely on soil, crops, and the environment.

Additionally, it also boosts cation exchange capacity and provides growth hormones like auxins [26]. Accordingly, these characteristics enhance its suitability as amendments in low-fertility soils. Hence the use of organic fertilizers has a greater relative economic benefit than mineral fertilization in newly reclaimed low-fertility sandy soils.

Biofertilizer contains beneficial microorganisms that promote plant growth by maintaining soil productivity and providing nutrients [27,28]. It assists in accessing nutrients such as nitrogen, phosphorus, and potassium from organic fertilizers, enhancing plant health and increasing salt and drought tolerance [29]. Biofertilizer is a promising approach to decreasing the use of conventional mineral fertilizers, particularly under current climate change [30,31]. Moreover, biofertilizer provides biodegradable substances, boosts soil stability and fertility, recycles nutrients, stimulates mycorrhiza symbiosis, mitigates soil contamination, develops bioremediation processes, and enhances biological control and antagonism of phytopathogenic organisms. Accordingly, applying biofertilizers increases plant growth, productivity, and fruit parameters and reduces mineral fertilization [32–34].

Biofertilizers application in combination with antioxidants gave a promotive effect on yield, fruit quality, and flesh oils content of the Chemlali olive cultivar [35]. Higher concentrations of N, K, and Na were found in the leaves of Chemlali olive trees after the application of biofertilizers rich in NPK. While olive trees treated with biofertilizers rich in NPK and Ca had higher chlorophyll concentrations. However, all foliar biofertilizer treatments led to a decrease in phenolic compounds in flower buds [36].

For these reasons, the present study aimed to investigate the combined effect of different organic and bio-fertilization sources on physiological parameters, leaf mineral contents, growth parameters, flowering, and fruit yield of kalamata olive trees grownunder low-fertility sandy soil in an arid environment.

2 Materials and Methods

2.1 Experimental Site and Agricultural Practices

A field experiment was conducted at El-Qantara, North Sinai, Egypt (30° 53′ 09″ N, 32° 05′ 04″ E) over two consecutive seasons (2020 and 2021). Tables 1 and 2 show that the experimental site has an arid climate, low precipitation, and sandy soil (83.92% sand, 7.36% clay, and 8.71% silt). Also, chemical analysis of irrigated water of the used well at the experimental site is presented in Table 3. Ten-year-old bearing Kalamata olive trees cultivated at 6 m × 7 m apart was used. Healthy trees having nearly uniform growth, vitality, and physical properties, a drip irrigation system, and recommended horticultural practices for growing olives were chosen for this study. The experimental design was factorial randomized complete block design with three replications for each treatment, and three trees represented each replication. The applied nitrogen for the olive tree was 250 g N/tree/year alongside the organic and biofertilizer applications.

Table 1: Climate variables of the experimental site during 2020 and 2021 as well as 35-yrs monthly averages (1987–2021)

	First season (2020)				Second season (2021)				35-yrs average (1987–2021)			
Month	Min	Max	RH	Perc	Min	Max	RH	Perc	Min	Max	RH	Perc
January	15.04	22.96	69.96	34.84	16.80	25.35	70.53	25.10	14.79	22.60	70.95	36.57
February	14.77	22.61	74.03	26.44	15.55	23.55	73.66	19.90	14.37	22.01	72.55	23.66
March	15.46	23.27	78.07	12.38	15.31	23.14	72.77	21.40	15.10	22.72	75.41	20.18
April	16.85	24.65	80.60	12.27	16.86	25.05	77.41	10.67	16.78	24.82	80.27	14.85
May	20.01	29.48	80.34	18.08	20.78	30.17	84.86	4.60	19.57	28.56	84.37	11.68
June	22.17	32.05	84.75	5.16	23.10	33.24	84.14	8.55	22.86	33.00	86.23	6.56
July	25.73	37.10	84.84	2.19	26.42	37.90	84.72	3.06	25.25	36.39	86.42	4.86

(Continued)

Table 1 (continued)

	First season (2020)				Second season (2021)				35-yrs average (1987–2021)			
Month	Min	Max	RH	Perc	Min	Max	RH	Perc	Min	Max	RH	Perc
August	26.61	38.15	83.11	2.45	27.65	39.79	82.01	1.63	26.12	37.56	84.45	2.27
September	26.52	38.22	82.15	1.88	25.46	36.75	75.67	5.55	24.84	35.92	78.63	11.55
October	24.65	35.60	73.79	13.76	23.20	33.77	71.72	22.16	22.55	32.98	74.45	15.28
November	21.01	30.90	67.24	19.22	21.55	31.60	74.75	20.56	19.63	29.15	71.26	22.07
December	18.68	27.71	67.77	26.62	17.23	26.07	70.02	37.68	16.59	25.09	70.57	26.03

Table 2: Physical and chemical properties of soil at the experimental site

Characteristic	Soil depth (cm)		
	(0–25)	(25–50)	(50–75)
Soil particles distribution			
Sand (%)	90.29	84.63	79.55
Clay (%)	5.20	6.44	7.75
Silt (%)	4.51	8.93	12.70
Soil texture	Loamy sand	Loamy sand	Sandy loam
Soluble cations and anions (meq/L)			
Calcium ($Ca^{2+)}$	6.72	7.95	12.37
Sodium (Na^{+})	8.67	11.28	13.37
Magnesium (Mg^{2+})	5.10	5.87	8.03
Potassium (K^{+})	0.40	0.36	0.35
Carbonate ($CO_3^{=}$)	–	–	–
Chloride (Cl^{-})	10.41	12.63	13.22
Bicarbonate (HCO_3^{-})	2.45	2.36	2.69
Sulfate ($SO_4^{=}$)	8.03	10.47	18.21
Electrical conductivity (dS/m)	2.89	2.45	3.38
pH	7.85	8.27	8.65
Organic matter (%)	0.17	0.12	0.08

Table 3: Chemical properties of the irrigated water of the used well at the experimental site

Characteristic	Value
Electrical conductivity (dS/m)	3.18
pH	7.4
Concentration (ppm)	2107

(Continued)

Table 3 (continued)

Characteristic	Value
Soluble cations and anions (meq/L)	
Sodium (Na^+)	15.05
Magnesium (Mg^{2+})	6.03
Calcium (Ca^{2+})	8.64
Potassium (K^+)	0.50
Bicarbonate (HCO_3^-)	2.62
Carbonate ($CO_3^=$)	–
Chloride (Cl^-)	22.26
Sulfate ($SO_4^=$)	5.34

2.2 Organic and Biofertilizer Applications

Two different types of organic fertilizers (goat manure and olive pomace) were examined. Organic fertilizers were brought from private goat farms and olive pomace from olive pressing factory. The chemical analysis of tested organic fertilizer materials is presented in Table 4. Goat manure and olive pomace fertilizers were used at one level for each (goat manure at 16.8 kg/tree/year, olive pomace at 8.5 kg/tree/year, and untreated trees (control). In mid-December of each season, the organic fertilizer sources were performed in two trenches dinged on both sides of the Kalamata olive tree at 40 cm depth and at 75 cm apart from the trunk and covered with trench soil.

Table 4: Chemical analysis of used organic fertilizer materials

Fertilizer	N (%)	P_2O_5 (%)	K_2O (%)	CaO (%)	MgO (%)
Goat manure	2.05	1.08	2.19	1.47	0.09
Olive pomace	2.08	1.19	1.28	0.45	0.25

These organic fertilizer materials were tested only or in combination with two types of bio-fertilizers [Nitrogen-fixing (N-fixing, contained *Azospirillum* spp. and *Azotobacter chroococcum*) bacteria at 150 g/tree, and amino acid mixture]. Bio-fertilizers used in the study were obtained from National Research Center, Giza, Egypt. The N-fixing bacteria fertilizer was applied in early March of each season, in trenches (45 cm length × 25 cm width × 20 cm depth) beneath the tree, whether organic fertilizers were provided or not. The amino acid mixture was applied from a commercial compound named protamine contains 16% free amino acids (alanine, arginine, aspartic, glutamic, glycine, histidine, leucine, lysine, serine, threonine, tryptophan, tyrosine, valine, and proline), 9.8% organic nitrogen, and 3.4% potassium oxide. The applied amino acid mixture was dissolved at 1.5% (v/v) and made up to one liter. It was then added to the soil under 'Kalamata' olive trees, where organic fertilizers under study were either added or not. The bio-fertilizers amino acid mixture was applied three times throughout the growing season three times at 70% full bloom (late April), 21 days after full bloom (fruit set stage), and a month later. In addition, non-fertilized trees were used as controls. A multiple of 81 uniform, healthy, and vigorous trees were selected [9 treatments (combinations of three organic fertilizer treatments with three bio-fertilizer treatments) × 3 replicates × 3 trees/replicate].

2.3 Measured Traits

2.3.1 Physiological Parameters

Ten mature leaves were collected from the middle of new shoot growth during late September of both seasons. Chlorophyll *a* and *b* and carotenoids were determined following the method of Fadeel [37]. Leaf samples were dried at 70°C until constant weight then ground to a powder for mineral content determination. A suitable sample (0.2 g) was taken from each dried ground leaf and wet digested using a mixture of Perchloric acid: Sulphuric acid (1:4 v/v) until a clear solution. Thereafter, in each leaf sample, the content of nitrogen (%), phosphorus (%), potassium (%), calcium (%), magnesium (%), and iron (ppm) was determined [38].

2.3.2 Growth, Yield, and Fruit Parameters

Tree canopy volume was measured using the following equation:

$$Tree\ canopy\ volume\ = \frac{1}{6}\pi D_1 \times D_2 \times (H_t - H_s)$$

where D_1 and D_2 are the diameters of the tree crown in two distinct directions, H_t is tree height, and H_s is the distance of the lowest point of the tree crown from the soil. Ten shoots were chosen randomly on each tree during full bloom (late April) and labeled to determine the number of flowers/inflorescences and number of inflorescences/shoots. The number of fruitlets and fruits was counted at monthly intervals up to harvest. The percentage of fruits set was determined using the following equation:

$$Fruit\ set\ percentage\ (\%) = \frac{Number\ of\ developed\ fruitlets}{Total\ initial\ number\ of\ flowers\ at\ full\ bloom} \times 100$$

Fruit retention was recorded based on the initial number of fruit set and the total number of fruitlet drop on the other during each season. Fruits harvesting was performed in November at the ripening stage, and 75% of olive fruits reached the violet skin color (the suitable stage for olive extraction). The fruits of each tree were weighed (kg). Fruit length (cm) and fruit width (cm) were recorded using a vernier caliper. Fresh weight (g) and stone weight (g) of olive fruits was determined for all fruit samples.

2.4 Statistical Analysis

R software (version 4.1.1) was used for performing all statistical analyses. The combined analysis of variance was applied to explore the differences among the organic fertilizer, biofertilizer, and their interactions in the two seasons. The differences among the assessed treatments were separated using the protected Tukey's HSD test at a significance level of $p \leq 0.05$. The heatmap was implemented to explore the relationship among evaluated traits in R software using RColorBrewer.

3 Results and Discussion

3.1 Physiological Parameters

The evaluated organic and bio-fertilizer applications and their interaction displayed significant impacts on most evaluated physiological parameters during the two seasons 2020 and 2021 (Tables 5 and 6). Both assessed organic fertilizers; olive pomace and goat manure substantially promoted photosynthetic pigments chlorophyll *a*, chlorophyll *b*, and carotenoids (Table 5) and leaf mineral contents N, P, K, Ca, Mg, and Fe (Table 6) compared with the non-fertilized control, with superiority of goat manure in both seasons. Likewise, both evaluated bio-fertilizer, N-fixing bacteria, and amino acid considerably elevated photosynthetic pigments and leaf mineral contents compared with untreated control with superiority of

N-fixing bacteria in both seasons (Tables 5 and 6). There was a significant interaction effect between organic and bio-fertilizers on all evaluated photosynthetic pigments and leaf mineral contents in both seasons. The lowest values in all evaluated photosynthetic pigments and leaf mineral contents were recorded by untreated control in both studied factors during both seasons (Tables 5 and 6). The interaction of goat manure treatment (16.8 kg/tree) with amino acids mixture (1.5%) displayed the highest enhancement in the content of chlorophyll *a*, chlorophyll *b*, and carotenoids in olive 'Kalamata' leaves by 24.86%, 52.18% and 85.59% respectively in the first season and 31.54%, 57.53% and 218.03% in the second season compared to the untreated control. Similarly, the treatment of goat manure combined with N-fixing bacteria improved the contents of N, P, K, Ca, Mg and Fe by 29.79%, 9.64%, 13.23%, 25.0%, 11.76%, and 26.85% in the first season and 16.77%, 6.74%, 8.99%, 28.57%, 26.67%, and 26.72% in the second season in comparison with untreated control trees.

Table 5: Influence of organic and bio-fertilizers on photosynthetic pigments of 'Kalamata' olive cv. during 2020 and 2021 growing seasons

Studied factor		Chlorophyll *a* (mg/100 g FW)		Chlorophyll *b* (mg/100 g FW)		Carotenoids (mg/100 g FW)	
		2020	2021	2020	2021	2020	2021
Organic fertilizer (O)							
Control		11.40^{b}	12.78^{b}	5.68^{b}	5.57^{c}	1.56^{b}	0.84^{b}
Olive pomace		12.24^{ab}	13.36^{b}	5.86^{b}	6.35^{b}	1.80^{a}	1.61^{a}
Goat manure		13.10^{a}	15.06^{a}	7.45^{a}	7.41^{a}	1.86^{a}	1.78^{a}
Bio-fertilizer (B)							
Control		11.81^{c}	13.05^{b}	5.92^{b}	6.08^{c}	1.82^{b}	1.22^{b}
Amino acid mixture		12.63^{a}	14.49^{a}	6.63^{a}	6.84^{a}	2.23^{a}	1.48^{a}
N-fixing bacteria		12.30^{b}	13.65^{ab}	6.46^{a}	6.52^{b}	2.17^{ab}	1.52^{a}
Interaction (O × B)							
Control	Control	11.10^{e}	12.11^{c}	5.27^{d}	5.11^{e}	1.18^{c}	0.61^{d}
	Amino acid mixture	11.84^{cde}	13.44^{abc}	5.91^{bcd}	6.41^{bc}	1.86^{abc}	0.88^{cd}
	N-fixing bacteria	11.27^{de}	13.30^{abc}	5.87^{bcd}	5.51^{de}	1.65^{bc}	1.03^{bcd}
Olive pomace	Control	11.60^{de}	12.79^{bc}	5.57^{cd}	6.05^{cde}	2.56^{ab}	1.44^{abc}
	Amino acid mixture	12.21^{bcd}	14.11^{abc}	5.96^{bcd}	6.08^{cd}	2.65^{ab}	1.64^{ab}
	N-fixing bacteria	12.90^{ab}	12.68^{bc}	6.07^{bcd}	6.94^{b}	3.20^{a}	1.77^{a}
Goat manure	Control	12.72^{bc}	14.37^{abc}	6.91^{bc}	7.07^{b}	1.72^{bc}	1.63^{ab}
	Amino acid mixture	13.86^{a}	15.93^{a}	8.02^{a}	8.05^{a}	2.19^{abc}	1.94^{a}
	N-fixing bacteria	12.73^{bc}	14.88^{ab}	7.43^{ab}	7.10^{b}	1.67^{bc}	1.77^{a}
ANOVA	**df**	***p*-value**					
Organic (O)	2	<0.001	<0.001	<0.001	<0.001	0.953	<0.001
Bio-fertilizer (B)	2	0.008	0.021	0.001	0.005	<0.001	<0.001
O × B	4	0.002	0.001	0.003	<0.001	0.038	0.001

Note: Means followed by different letters under the same factor are significantly different according to Tukey's HSD test ($p \leq 0.05$).

Table 6: Influence of organic and bio-fertilizers on leaf mineral contents of 'Kalamata' olive cv. during 2020 and 2021 growing seasons

Studied factor		N (%)		P (%)		K (%)		Ca (%)		Mg (%)		Fe (ppm)	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Organic fertilizer (O)													
Control		1.58^{a}	1.66^{a}	0.83^{a}	0.89^{a}	1.94^{a}	1.96^{a}	0.51^{a}	0.52^{b}	0.17^{b}	0.17^{b}	16.71^{b}	16.17^{b}
Olive pomace		1.73^{a}	1.74^{a}	0.86^{a}	0.90^{a}	1.96^{a}	1.99^{a}	0.56^{a}	0.52^{b}	0.19^{a}	0.19^{a}	15.51^{c}	15.18^{b}
Goat manure		1.86^{a}	1.85^{a}	0.93^{a}	0.98^{a}	2.15^{a}	2.07^{a}	0.56^{a}	0.58^{a}	0.19^{a}	0.19^{a}	19.00^{a}	18.72^{a}
Bio-fertilizer (B)													
Control		1.62^{b}	1.67^{c}	0.87^{ab}	0.92^{a}	1.94^{b}	1.94^{b}	0.53^{b}	0.52^{b}	0.17^{c}	0.17^{c}	16.29^{b}	15.93^{b}
Amino acid mixture		1.73^{a}	1.75^{b}	0.85^{b}	0.91^{a}	1.99^{b}	1.97^{b}	0.56^{a}	0.56^{a}	0.18^{b}	0.19^{b}	18.61^{a}	18.10^{a}
N-fixing bacteria		1.81^{a}	1.82^{a}	0.90^{a}	0.95^{a}	2.12^{a}	2.11^{a}	0.53^{b}	0.54^{b}	0.19^{a}	0.20^{a}	16.32^{b}	16.03^{b}
Interaction (O × B)													
Control	Control	1.41^{b}	1.55^{b}	0.83^{a}	0.89^{a}	1.89^{b}	1.89^{b}	0.48^{c}	0.49^{c}	0.17^{c}	0.15^{d}	16.46^{bcd}	15.98^{bcd}
	Amino acid mixture	1.66^{ab}	1.73^{ab}	0.83^{a}	0.90^{a}	1.96^{ab}	1.95^{b}	0.52^{bc}	0.54^{bc}	0.18^{bc}	0.18^{c}	17.05^{bcd}	16.16^{bcd}
	N-fixing bacteria	1.66^{ab}	1.70^{ab}	0.83^{a}	0.90^{a}	1.97^{ab}	2.05^{ab}	0.53^{bc}	0.53^{bc}	0.18^{bc}	0.18^{c}	16.63^{bcd}	16.38^{bcd}
Olive pomace	Control	1.74^{ab}	1.74^{ab}	0.88^{a}	0.92^{a}	1.92^{b}	1.97^{b}	0.54^{abc}	0.52^{bc}	0.18^{bc}	0.18^{c}	14.88^{bc}	14.39^{cd}
	Amino acid mixture	1.69^{ab}	1.72^{ab}	0.80^{a}	0.88^{a}	1.87^{b}	1.91^{b}	0.57^{ab}	0.52^{bc}	0.18^{bc}	0.19^{bc}	17.93^{ab}	17.90^{ab}
	N-fixing bacteria	1.76^{ab}	1.76^{ab}	0.89^{a}	0.90^{a}	2.09^{ab}	2.10^{ab}	0.56^{ab}	0.52^{bc}	0.19^{ab}	0.20^{ab}	13.72^{d}	13.25^{d}
Goat manure	Control	1.71^{ab}	1.73^{ab}	0.89^{a}	0.95^{a}	2.02^{ab}	1.97^{b}	0.56^{ab}	0.56^{b}	0.18^{bc}	0.18^{c}	17.53^{bc}	17.44^{bc}
	Amino acid mixture	1.83^{a}	1.81^{ab}	0.91^{a}	0.95^{a}	2.14^{ab}	2.06^{ab}	0.60^{a}	0.63^{a}	0.19^{ab}	0.19^{bc}	20.88^{a}	20.25^{a}
	N-fixing bacteria	2.00^{a}	2.01^{a}	0.99^{a}	1.04^{a}	2.29^{a}	2.19^{a}	0.51^{bc}	0.56^{b}	0.20^{a}	0.21^{a}	18.61^{ab}	18.47^{ab}
ANOVA	**df**	***p*-value**											
Organic (O)	2	0.059	0.151	0.166	0.072	0.338	0.085	0.140	0.005	0.001	0.030	0.001	<0.001
Bio-fertilizer (B)	2	0.011	0.019	0.042	0.124	0.009	0.011	0.001	0.030	0.001	0.002	0.002	<0.001
O × B	4	0.031	0.025	0.369	0.096	0.102	0.021	0.004	0.002	0.108	0.005	<0.001	<0.001

Note: Means followed by different letters under the same factor are significantly different according to Tukey's HSD test ($p \leq 0.05$).

3.2 Growth, Yield, and Fruit Parameters

Tree canopy volume, number of flowers per inflorescence, number of inflorescences per shoot, initial fruit set, fruit retention, fruit length, fruit width, fruit fresh weight, and total fruit yield significantly differed between the tested treatments and their interaction in both seasons (Tables 7 and 8). The evaluated organic fertilizers, olive pomace, and goat manure substantially ameliorated all the parameters mentioned above compared with that of the non-fertilized trees with the superiority of goat manure (16.8 kg/tree/year) in most traits in the two seasons of 2020 and 2021. Similarly, the assessed bio-fertilizers; N-fixing bacteria, and amino acid mixture considerably enhanced all growth, yield, and fruit parameters compared to the untreated control, with the superiority of N-fixing bacteria in most traits (Tables 7 and 8). The lowest values in all studied growth, yield, and fruit parameters were produced by the untreated trees in both studied factors in both seasons (Tables 7 and 8). On the contrary, goat manure combined with N-fixing bacteria presented the uppermost interaction impact compared to the other treatments in both seasons. The interaction between goat manure with N-fixing bacteria improved tree canopy volume, number of flowers per inflorescence, number of inflorescences per shoot, initial fruit set, and fruit retention, by 48.08%, 68.10%, 179.1%, 91.13%, and 111.2% respectively in the first season and 26.06%, 92.06%, 117.5%, 19.68%, and 104.0% compared untreated control. Similarly, the interaction between goat manure with N-fixing bacteria enhanced fruit length, fruit width, fruit fresh weight, and total fruit yield by 31.60%, 22.84%, 53.35%, and 86.56% respectively in the first season and 18.60%, 24.68%, 59.53%, and 70.73% compared to untreated trees.

Table 7: Influence of organic and bio-fertilizers on growth parameters of 'Kalamata' olive cv. during the 2020 and 2021 growing seasons

Studied factor		Tree canopy (m)		No. of inflorescences/ shoot		No. of flowers/ inflorescence		Initial fruit set (%)		Fruit retention (%)	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Organic fertilizer (O)											
Control		10.24^{b}	10.50^{b}	14.02^{c}	17.08^{b}	94.64^{c}	69.91^{c}	18.00^{b}	25.97^{b}	3.00^{b}	2.55^{c}
Olive pomace		10.68^{b}	11.07^{ab}	17.24^{b}	21.15^{b}	109.01^{b}	83.69^{b}	19.50^{b}	26.15^{b}	3.23^{b}	3.04^{b}
Goat manure		12.05^{a}	12.47^{a}	25.56^{a}	28.23^{a}	123.32^{a}	95.19^{a}	25.52^{a}	27.61^{a}	4.57^{a}	3.70^{a}
Bio-fertilizer (B)											
Control		10.11^{c}	11.15^{b}	16.21^{b}	18.86^{b}	98.84^{b}	76.08^{c}	18.24^{c}	25.75^{b}	3.14^{b}	2.80^{b}
Amino acid mixture		11.01^{b}	11.45^{a}	22.31^{a}	24.71^{a}	121.65^{a}	82.60^{b}	20.13^{b}	25.44^{b}	3.79^{a}	2.97^{b}
N-fixing bacteria		11.86^{a}	11.44^{a}	18.29^{b}	22.88^{a}	106.48^{b}	90.12^{a}	24.65^{a}	28.54^{a}	3.88^{a}	3.52^{a}
Interaction (O × B)											
Control	Control	9.36^{d}	9.90^{c}	9.11^{e}	13.41^{c}	69.96^{c}	54.41^{e}	14.99^{d}	24.59^{bc}	2.14^{e}	2.00^{c}
	Amino acid mixture	10.37^{bdc}	10.69^{c}	18.18^{cd}	19.53^{abc}	111.55^{b}	78.30^{cd}	19.32^{cd}	26.10^{bc}	3.45^{cd}	2.72^{bc}
	N-fixing bacteria	10.99^{bc}	10.92^{c}	14.76^{de}	18.30^{bc}	102.40^{b}	77.01^{d}	19.71^{cd}	27.23^{ab}	3.41^{cd}	2.93^{b}
Olive pomace	Control	10.17^{cd}	11.22^{abc}	17.32^{cd}	17.56^{bc}	105.75^{b}	81.99^{bcd}	18.02^{cd}	25.52^{bc}	2.98^{d}	2.73^{bc}
	Amino acid mixture	11.17^{bc}	11.06^{bc}	19.71^{bcd}	24.69^{ab}	121.90^{ab}	80.29^{bcd}	14.90^{d}	23.96^{c}	3.03^{d}	2.86^{bc}
	N-fixing bacteria	10.72^{bc}	10.94^{c}	14.70^{de}	21.19^{abc}	99.39^{b}	88.80^{bcd}	25.58^{ab}	28.96^{a}	3.70^{bc}	3.54^{ab}
Goat manure	Control	10.80^{bc}	12.34^{ab}	22.20^{bc}	25.62^{ab}	120.80^{ab}	91.84^{b}	21.71^{bc}	27.14^{ab}	4.30^{ab}	3.65^{ab}
	Amino acid mixture	11.49^{b}	12.60^{a}	29.05^{a}	29.92^{a}	131.50^{a}	89.20^{bc}	26.19^{ab}	26.25^{bc}	4.90^{a}	3.35^{ab}
	N-fixing bacteria	13.86^{a}	12.48^{a}	25.43^{ab}	29.16^{a}	117.65^{ab}	104.55^{a}	28.65^{a}	29.43^{a}	4.52^{a}	4.08^{a}
ANOVA	**df**	***p*-value**									
Organic (O)	2	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.032
Bio-fertilizer (B)	2	<0.001	<0.001	0.033	0.002	<0.001	<0.001	<0.001	0.003	0.005	0.015
O × B	4	0.035	0.047	0.016	0.021	0.036	0.004	0.036	0.004	0.009	0.002

Note: Means followed by different letters under the same factor are significantly different according to Tukey's HSD test ($p \leq 0.05$).

Table 8: Influence of organic and bio-fertilizers on yield, and fruit parameters of 'Kalamata' olive cv. during the 2020 and 2021 growing seasons

Studied factor		Fruit length (cm)		Fruit width (cm)		Fruit fresh weight (g)		Stone weight (g)		Total fruit yield (kg/tree)	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Organic fertilizer (O)											
Control		2.54^{b}	2.58^{b}	1.73^{a}	1.66^{a}	3.96^{c}	3.55^{b}	1.03^{a}	0.75^{a}	33.09^{c}	64.76^{b}
Olive pomace		2.75^{a}	2.75^{a}	1.83^{a}	1.75^{a}	4.39^{b}	3.79^{ab}	1.19^{a}	0.78^{a}	34.23^{b}	68.51^{b}
Goat manure		2.92^{a}	2.74^{a}	1.89^{a}	1.81^{a}	4.78^{a}	4.25^{a}	1.20^{a}	0.85^{a}	44.48^{a}	89.27^{a}
Bio-fertilizer (B)											
Control		2.61^{b}	2.62^{b}	1.74^{c}	1.66^{b}	4.36^{b}	3.48^{b}	1.06^{a}	0.77^{a}	33.19^{b}	68.65^{b}
Amino acid mixture		2.76^{a}	2.66^{b}	1.82^{b}	1.74^{ab}	4.36^{ab}	3.99^{a}	1.16^{a}	0.81^{a}	37.86^{a}	78.01^{a}
N-fixing bacteria		2.84^{a}	2.79^{a}	1.89^{a}	1.82^{a}	4.60^{a}	4.12^{a}	1.20^{a}	0.79^{a}	40.75^{a}	75.88^{a}
Interaction (O × B)											
Control	Control	2.31^{c}	2.42^{b}	1.62^{c}	1.58^{b}	3.28^{c}	2.99^{c}	0.91^{a}	0.72^{a}	26.19^{d}	52.20^{c}
	Amino acid mixture	2.61^{b}	2.63^{ab}	1.74^{bc}	1.69^{b}	4.15^{b}	3.67^{abc}	1.08^{a}	0.78^{a}	35.77^{bc}	71.90^{b}
	N-fixing bacteria	2.71^{b}	2.70^{a}	1.84^{abc}	1.71^{b}	4.46^{ab}	3.99^{abc}	1.09^{a}	0.76^{a}	37.31^{bc}	70.19^{b}
Olive pomace	Control	2.67^{b}	2.72^{a}	1.77^{abc}	1.69^{b}	4.51^{ab}	3.40^{bc}	1.10^{a}	0.78^{a}	31.80^{cd}	66.49^{bc}
	Amino acid mixture	2.80^{ab}	2.73^{a}	1.88^{ab}	1.78^{ab}	4.38^{ab}	4.38^{ab}	1.19^{a}	0.79^{a}	34.81^{bcd}	70.72^{b}
	N-fixing bacteria	2.79^{ab}	2.80^{a}	1.83^{abc}	1.78^{ab}	4.30^{ab}	3.59^{abc}	1.27^{a}	0.78^{a}	36.06^{bc}	68.33^{b}
Goat manure	Control	2.84^{ab}	2.73^{a}	1.84^{abc}	1.71^{b}	4.77^{ab}	4.06^{abc}	1.18^{a}	0.83^{a}	41.59^{ab}	87.28^{a}
	Amino acid mixture	2.88^{ab}	2.64^{ab}	1.83^{abc}	1.76^{ab}	4.55^{ab}	3.92^{abc}	1.19^{a}	0.88^{a}	43.01^{ab}	91.40^{a}
	N-fixing bacteria	3.04^{a}	2.87^{a}	1.99^{a}	1.97^{a}	5.03^{a}	4.77^{a}	1.23^{a}	0.83^{a}	48.86^{a}	89.12^{a}
ANOVA	**df**	***p*-value**									
Organic (O)	2	0.001	0.025	0.079	0.082	<0.001	<0.001	0.144	0.532	<0.001	0.001
Bio-fertilizer (B)	2	0.021	0.002	0.001	0.001	<0.001	0.003	0.412	0.315	0.003	0.002
O × B	4	0.008	0.016	0.036	0.004	0.007	0.006	0.229	0.245	0.005	0.021

Note: Means followed by different letters under the same factor are significantly different according to Tukey's HSD test ($p \leq 0.05$).

3.3 Interrelationship among the Assessed Applications and Evaluated Parameters

Understanding the interrelationship between the evaluated applications and studied parameters of 'Kalamata' olive trees is a pivotal aspect that can provide helpful information. The heatmap and hierarchical clustering is an applicable statistical analysis to explore the relationship between the evaluated applications and studied parameters for 'Kalamata' olive cv. The present study divided the evaluated organic and bio-fertilizers into clusters based on photosynthetic pigments, leaf mineral contents, growth, yield, and fruit parameters (Fig. 1). The combination treatment of goat manure with N-fixing bacteria and amino acid mixture exhibited the highest values, followed by olive pomace with both biofertilizers for all evaluated parameters (depicted in blue color). On the contrary, the non-fertilized control olive trees possessed the lowest values of all evaluated parameters (represented in red color).

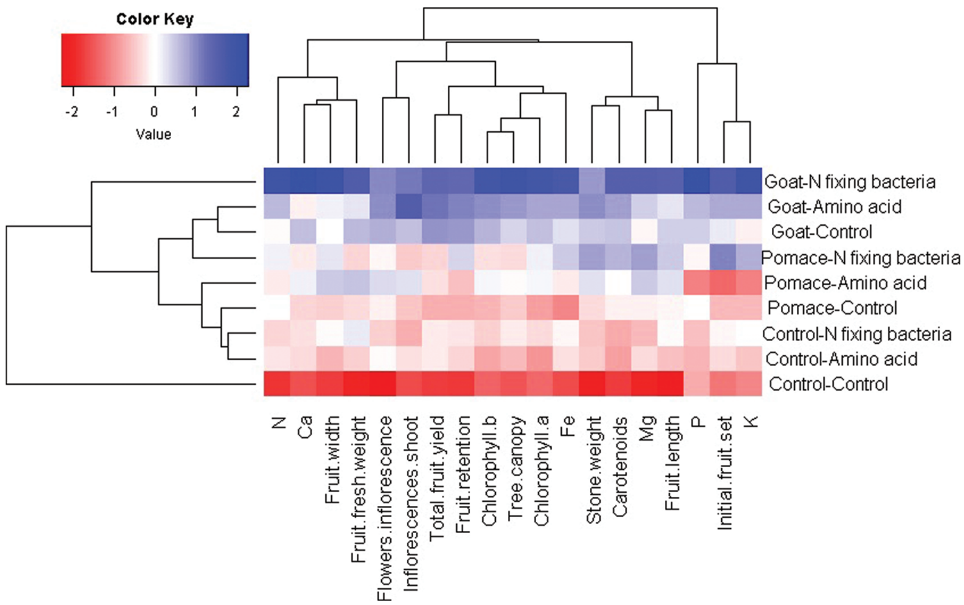

Figure 1: Heatmap and hierarchical clustering divide the evaluated organic and bio-fertilizers and amino acid mixture into clusters based on photosynthetic pigments, leaf mineral contents, growth, yield, and fruit parameters. Red and blue colors reveal low and high values for the corresponding parameters, respectively

4 Discussion

Newly reclaimed soils suffer from a deficiency of mineral nutrients and numerous environmental stresses [39,40]. Mineral fertilization contributes to environmental pollution with nitrate leading to negative impacts on human health [41]. Consequently, it is necessary to find economic and environment-friendly applications to ameliorate plant growth and productivity of 'Kalamata' olive trees under these poor conditions [42,43]. Organic and biofertilizers have become more critical to sustain agricultural production and diminish mineral fertilization. Furthermore, in a circular economy framework, using waste by-products as fertilizer is essential to support sustainable agriculture [44,45]. Goat manure is conventional manure collected from local large goat herds. Besides, olive pomace is obtained from olive oil production and could be used as a beneficial alternative organic fertilizer. This is consistent with the findings of Erel et al. [46] and Tekaya et al. [47]. Similarly, Bellitürk et al. [48] showed that olive 'Kalamata' trees mineral elements (N, P, K, Ca, Mg, and Fe) were increased by utilizing organic fertilizers. Hence, the current study was performed to explore the influence of integrated use of organic (olive pomace and goat manure) and bio-fertilizer N-fixing bacteria as well as amino acid on physiological growth, yield, and fruit parameters of 'Kalamata' olive trees grown on poor-fertility sandy soil containing low nutrient contents. Organic material provides a source of phosphorus and carbon to the microflora population, as well as sites for the microflora to colonize. Bacteria secrete enzymes that catalyze the liberation of calcium and phosphorus from insoluble calcium phosphate and iron and phosphorus from insoluble iron phosphate [49]. As a result, it supplies the amount of calcium required to maintain the stability and integrity of the cytoplasmic membranes in the table olive fruit, by enhancing firmness fruit quality and boosting the cell wall's resistance by forming bonds with the median lamella's pectin. Calcium concentrations have been linked to physiological processes such as fruit softening, browning reactions, and ripening-related alterations [50].

Organic fertilizer greatly benefits poor-fertility sandy soils by elevating soil organic matter, water-holding capacity, and providing a source of slow-release macro and micronutrients [51]. It improves cation exchange capacity and elevates soil fertility, soil structure, and moisture retention [52]. Earlier published reports disclosed the importance of organic fertilization in elevating nutrient contents (nitrogen, phosphorus, potassium, calcium, and magnesium) in the soil [53–55].

Moreover, it stimulates beneficial soil micro-organisms that have a crucial role in improving soil properties, adjusting undesirable fluctuations of soil pH and processing ability, and mobilizing unavailable forms of nutrient components [56,57]. Accordingly, these advantages ameliorate plant growth and productivity under poor-fertility sandy soils. The obtained results displayed that both evaluated organic fertilizers; goat manure (16.8 kg/tree/year) and olive pomace (8.5 kg/tree/year), significantly promoted photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) of 'Kalamata' olive treated trees. Also, these organic fertilizers under study significantly increased leaf mineral contents (N, P, K, Ca, Mg, and Fe), tree canopy volume, number of flowers per inflorescence, number of inflorescences per shoot, initial fruit set, fruit retention, fruit length, fruit width, fruit fresh weight, and total fruit yield compared with the non-fertilized kalamata olive trees. The enhancement of evaluated parameters was attributed to the improved availability of nutrients released in the soil by olive pomace and goat manure compared to the non-fertilized olive trees. Besides, olive pomace and goat manure contain an adequate amount of organic matter which holds water and enhances the mineralization process. This rise in physical characteristics could be brought about by the increased leaf area of kalamata olive trees after using organic and bio-fertilizers. Under the application of organic and bio-fertilizers, there was a large increase in leaf area and stomatal density, which may have been caused by the growth in cell size and number. This may also be a result of the synergistic and antagonistic interactions between cytokines and auxins, which are both facilitated by all treatments with biofertilizers under investigation and implicated in the control of cell expansion [58].

In this context, El-Alakmy et al. [59] depicted that applying organic fertilizer particularly fish scrap provided a high olive yield and quality and protected trees from environmental stress. Similarly, Nwaigwe et al. [60] elucidated that organic fertilizer, particularly goat manure stimulated sweet potato growth, yield component, and root yield compared to non-fertilized control. Moreover, Hassan et al. [61] proved that organic fertilizer, especially poultry manure, improved flowering density, fruit set, fruit yield, and quality of olive compared to the non-fertilized control.

Biofertilizer is a natural stimulant that contains effective microbial strains [62]. It enhances soil fertility and plant nutrient uptake by increasing the availability of nutrients to plant roots [27]. Hence, it stimulates plant growth and productivity. The application of biofertilizer reduces synthetic fertilizer's utilization and mitigates its hazardous impacts [63]. Therefore, its application enhances sustainable agriculture, remediation of polluted soils, and improves the quality of agricultural products [64]. It is an effective, promising, renewable source, inexpensive, and eco-friendly alternative to mineral fertilization. The present study investigated the impact of bio-fertilizer: N-fixing bacteria, and amino acid mixture on Kalamata olive photosynthetic pigments, leaf mineral contents, growth, yield, and fruit parameters. Both evaluated N-fixing bacteria and amino acid mixture significantly enhanced studied parameters compared with the untreated trees.

Similarly, Senjobi et al. [51,65] disclosed that applied organic fertilizer and supported with biofertilizer remarkably enhanced photosynthetic pigments and mineral contents of N, P, K, Ca, Mg, Fe, Mn, and Zn.

Furthermore, Mazeh et al. [66] deduced that applying organic fertilizer combined with biofertilizer is highly efficient in supplying the nutritional requirements of olive trees with essential elements. These nutrients are vital compounds for the biosynthesis of nucleic acids, enzymes, proteins, and photosynthetic pigments [67,68]. Additionally, these nutrients are valuable compounds for transferring and storing energy in plants and maintaining cell turgor, cell expansion, and stomatal function [69,70]. Accordingly, all these positive impacts of organic fertilizer and biofertilizer are reflected in photosynthetic pigments, leaf mineral contents, growth, yield, and quality parameters of olive.

The heatmap and hierarchical clustering is a proper statistical approach to exploring the association between assessed treatments and studied parameters [71,72]. It was employed in the current study to explore the interrelationship among the evaluated organic and biofertilizer N-fixing bacteria and amino acid mixture based on photosynthetic pigments, leaf mineral contents, growth, yield, and fruit parameters. The evaluated applications were divided into different clusters based on their performance. The application of goat manure combined with N-fixing bacteria or amino acid mixture exhibited the highest values, followed by olive pomace combined with both N-fixing bacteria and amino acid mixture for all evaluated parameters compared with the non-fertilized control trees. Accordingly, the heatmap and hierarchical clustering results reinforced the aforementioned presented results. Similarly, El-Alakmy et al. [59,73] and Nwaigwe et al. [60,74] effectively applied the heatmap and hierarchical clustering to explore the relationship between studied treatments and evaluated traits.

5 Conclusion

The present study clarified the potential of organic fertilizers (goat manure or olive pomace) combined with biofertilizers (N-fixing bacteria and amino acid mixture) in enhancing photosynthetic pigments (chlorophyll a, b, and carotenoids), leaf mineral contents (N, P, K, Ca, Mg, and Fe), tree canopy volume, number of flowers per inflorescence, number of inflorescences per shoot, initial fruit set, fruit retention, fruit length, fruit width, fruit fresh weight, and total fruit yield of 'Kalamata' olive cultivar compared to the non-fertilized control trees. Conclusively, the co-application of organic and biofertilizer, particularly goat manure (16.8 kg/tree/year) combined with N-fixing bacteria (150 g/tree of *Azospirillum* spp. and *Azotobacter chroococcum*) could be recommended in low-fertility soil for sustaining olive production and reducing mineral fertilization. Agricultural byproducts can be used efficiently in the production of good and affordable organic fertilizers, which can then be used to solve the problems of safe disposal of agricultural wastes in Egypt, in order to increase the productivity of one of the most significant dual-purpose crops in Egypt and the rest of the world, olive trees 'Kalamata' cv.

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